

Short Communication

Histological Transmogrification in Organs of *Heterobranchus bidorsalis* (É. Geoffroy Saint-Hilaire, 1809) Juveniles Exposed To 2-[(Phosphonomethyl) amino] acetic acid

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Abstract

Static bioassay was conducted to determine the 96h median lethal level (LC₅₀) of 2-[(Phosphonomethyl) amino] acetic acid on *Heterobranchus bidorsalis* juveniles (4.90 ± 0.13 g and length of 5.02 ± 1.0 cm respectively) and to describe the histological changes in the gill and liver. LC₅₀ of *H. bidorsalis* fingerlings was determined graphically as 18.07 mg/ L⁻¹. Fish displayed the following behaviors respiratory distress (hyperventilation), erratic swimming, jumping out of the tank at higher concentration, molting, discolouration, irregular operculum opening and tail beat frequencies, loss of reflex and settling of the experimental fish at the bottom of the aquaria at higher concentration. Also fish mortality at varying concentrations increased with increasing concentration of glyphosate herbicides. Histological examination revealed mild degeneration in the gill architecture, atrophy of the filament artery, distorted lamellar epithelium, epithelia proliferation/erosion of gill filaments and lamellae and necrosis of the gill cells. The liver showed no noticeable lesion at lower concentration of 2-[(Phosphonomethyl) amino] acetic acid, slight degeneration and vacuolation of the hepatocyte, evidence of inflammation of the liver cells with cellular infiltration of hepatocellular parenchyma and evidence of fatty acid degeneration and severe hepatic damage as a result of hepatotoxicity of 2-[(Phosphonomethyl) amino] acetic acid.

INTRODUCTION

With the advent of the Green Revolution in the second half of the 20th century, farmers began to use technological advances to boost yields; synthetic fertilizers, pesticides and herbicides [1]. When herbicides are applied, they have the potential to run off and that could impact the waters used in aquaculture thereby causing massive fish death. Global aquaculture production has increased geometrically, at an average rate of 8.8% per year [2] and the most popular culturable fish species in Nigeria and most parts of Africa are the member of the catfish family called clariidae. *Heterobranchus bidorsalis*, the African catfish is an air breathing catfish found in Africa [3]. They can grow to a remarkable big size, 14.0 kg [4]. It occupies a unique prominent position in commercial fisheries in Nigeria and is the second most

important clariid catfish in Africa next to *Clarias griepinus* [5-7].

2-[(Phosphonomethyl) amino] acetic acid commonly known as glyphosate are broad-spectrum systemic herbicide used to kill weeds, especially grasses and annual broadleaf weeds known to compete with crops of commercial value grown around the globe [8]. The residue of this herbicide had been found at phyto-toxic concentration in lakes, streams and ground water, which resulted from run-off from treated field [9]. The unpleasing development of this to fish as a result of run-off from treated fields to streams, ground water, rivers, lakes etc. is yet to be fully investigated and evaluated [10]. It is a known fact that the aquatic environment is a sink to toxic contaminants which finds their way to the water bodies through agricultural, domestic and industrial activities [11].

This study is therefore aimed at determining the median lethal concentration (LC₅₀) of *Heterobranchus bidorsalis* juveniles exposed to varying concentrations of glyphosate and also to determine the effect these varying concentrations on the histology of gills and liver.

MATERIAL AND METHODS

Two hundred and fifty apparently healthy *Heterobranchus bidorsalis* juveniles from mixed sex and the same brood Stock, mean weight, 4.90 ± 0.13g and average length of 5.02 ± 1.0 cm respectively were procured from Akin Sateru Farms (a private hatchery) in Lagos State and transported live to Federal College of Fisheries and Marine Technology laboratory, Victoria Island, Lagos. They were later stocked in aquaria where they are allowed to acclimatize for 7 days.

Ten *H. bidorsalis* juveniles (4.90 ± 0.13 g) were stocked into each aquarium (40 cm x 29 cm x 28 cm), with three replicates per treatment. 2-[(Phosphonomethyl) amino] acetic acid (Glyphosate herbicide) was obtained from an Agro- allied shop in Victoria Island, Lagos State, Nigeria. The treatments were: Treatment 1, 9.6mg 2-[(Phosphonomethyl) amino] acetic acid /L⁻¹ of water; Treatment 2, 14.4 mg 2-[(Phosphonomethyl) amino] acetic acid /L⁻¹ of water; Treatment 3, 19.2 mg 2-[(Phosphonomethyl) amino] acetic acid /L⁻¹ of water; Treatment 4, 21.6 mg 2-[(Phosphonomethyl) amino] acetic acid / L⁻¹ of water, Treatment 5, 24.0 mg 2-[(Phosphonomethyl) amino] acetic acid / L⁻¹ of water and Control , 0 g 2-[(Phosphonomethyl) amino] acetic acid / L⁻¹ of water. Standard methods [12], were employed in carrying out the experiment. Prior to the commencement of the experiment, the fish were starved for 2 days to minimize the amount of waste in the test media and to prevent organic decomposition and oxygen depletion. The experiment was conducted under standard static bioassay conditions. Temperature, pH, dissolved oxygen, and

conductivity level were determined using standard methods and readings were taken at 24 h interval for 96 h. At the end of the treatment period, two fish from each treatment tank were removed, weighed, killed by decapitation and vital organs such as the gill and liver were removed, fixed for 24 h in formalin-saline solution made of equal volumes of 10% formalin and 0.9% NaCl solution. Histological sections of 7µm thickness were prepared following standard procedures [13] photomicrographs were taken with Leitz (Ortholux) microscope and camera.

RESULTS

The following behaviors were exhibited during the definitive test; respiratory distress (hyperventilation), erratic swimming, jumping out of the tank (at higher concentration), molting, discolouration, irregular operculum and tail beat frequencies, loss reflex and weak and settling at the bottom of the aquaria at higher concentration. Fish mortality at varying concentrations increased with increasing concentration of glyphosate herbicides, 17% - 92% (Table 1). All fish in the control treatment survived throughout the 96 hours duration of the experiment. The cumulative mortalities and acute 96h LC₅₀ of glyphosate on juveniles of *Heterobranchus bidorsalis* was determined according to Behrens-Karber's method [14]. There were significant losses of fish with increase in glyphosate herbicides concentration (P < 0.05). The LC₅₀ was recorded graphically at 18.07 mg 2-[(Phosphonomethyl) amino] acetic acid /L⁻¹ of water (Figure 1).

Histological changes in the organs (gills and livers) of *H. bidorsalis* juveniles were represented in Table (3).

DISCUSSION

In this study, *H. bidorsalis* juveniles exposed to 2-[(Phosphonomethyl) amino] acetic acid showed some behavioural changes which are stress induced namely, respiratory distress (hyperventilation), erratic swimming, jumping out of the

Table 1: Mortality (%) of *Heterobranchus bidorsalis* juveniles exposed to different concentrations of 2-[(Phosphonomethyl) amino] acetic acid (glyphosate herbicide).

Concentrations(mg/ l ⁻¹)	Time (Hours)				
	0	24	48	72	96
0	0.0 ± 0.0	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
9.6	0.0 ± 0.0	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
14.4	0.0 ± 0.0	0.0 ± 0.00	0.0 ± 0.00	8.0 ± 0.17	17.0 ± 0.05
19.2	0.0 ± 0.0	8.0 ± 0.20	17.0 ± 0.00	42.0 ± 0.31	58.0 ± 0.11
21.6	0.0 ± 0.0	17.0 ± 0.03	33.0 ± 0.20	58.0 ± 0.04	75.0 ± 0.05
24.0	0.0 ± 0.0	50.0 ± 0.15	58.0 ± 0.13	67.0 ± 0.10	92.0 ± 0.02

Table 2: The lethal concentrations (LC₅₀) values of 2-[(Phosphonomethyl) amino] acetic acid (glyphosate herbicide) to *H. bidorsalis* juveniles after several hours.

Time (Hours)	Log C Value LC ₅₀	LC ₅₀ (mg/l)
24	1.387	24.38mg/l
48	1.366	23.23mg/l
72	1.318	20.80mg/l
96	1.257	18.07mg/l

tank (at higher concentration), molting, discolouration, irregular operculum opening and tail beat frequencies, loss of reflex and settling of the experimental fish at the bottom of the aquaria at higher concentration. In a related study Ayoola [15] reported similar behavioural changes for *Oreochromis niloticus* juvenile subjected to glyphosate herbicide toxicity. Kulakkattolickal and Kramer [16] also corroborated this report that all the afore mentioned behavioral changes are normal observation in acute and chronic toxicity test. Also, the behavioural changes noticed in this study are signals to respiratory impairment [17], and this may be as a result of the effect of glyphosate on the gills.

Also fish mortality at varying concentrations increased with increasing concentration of glyphosate herbicides. Mortality (%) of *H. bidorsalis* fingerlings exposed to different concentrations of 2-[(Phosphonomethyl) amino] acetic acid (Table 3) showed that the fish were sensitive to concentrations from 14.4 – 24.0 mg L⁻¹. The table indicated that within 96h, about 17% of the fish died in concentration, 14.4mg L⁻¹, while 58% died in concentration, 19.2mg L⁻¹, 75% died in concentration 21.6mg L⁻¹ and finally, 92% died in concentration 24.0mg L⁻¹. This report is in tandem with that of Dahunsi and Oranusi [18] who reported that the mortality of *Clarias gariepinus* increased as the concentrations of synthetic resin effluent increases also, Rzymski et al., [19] reported a significant decrease in tax number of benthic invertebrates between the control and treatments (glyphosate based herbicide).

The concentration values were converted to Logit, while the mortality (%) was converted to Probit values according to methods of Hewlett & Plackett [20], and the transformed values were used to determine the 96h LC₅₀ graphically. Figure (1) presents the LC₅₀ graph with the regression equation $Y = 10.233x - 7.8583$, where $y =$ probit response and $x =$ logit (log-dose). From the equation, the 96h LC₅₀ was calculated as 18.07 mg L⁻¹. This result is at variance with that of Langiano and Martinez [21], they reported LC₅₀ of 13.69mgL⁻¹ for Neotropical fish *Prochodus lineatus* subjected to glyphosate herbicide.

Histological changes observed in the gill and liver of *Heterobranchus bidorsalis* juveniles in this study showed varying degrees of alterations (Table 3). In *H. bidorsalis* juveniles exposed to 0ml (control) 2-[(Phosphonomethyl) amino] acetic acid toxicity, the gill histology showed normal gill architecture [22,23]. This study is in tandem with that reported by Abalaka [24]. Also increase in the concentration of 2-[(Phosphonomethyl) amino] acetic acid showed diverse histological changes with frequencies of alterations increasing as the time of exposure increases. In this study, [25] the key changes in the histology gills of *H. bidorsalis* juveniles exposed to 2-[(Phosphonomethyl) amino] acetic acid are degeneration in gill architecture, atrophy of the filament artery, erosion of the gill filament and lamellae and necrosis of gill cells. Ayoola [26] in a study on the effect of glyphosate on *Oreochromis niloticus* juveniles corroborated these changes by reporting oedema, epithelia lifting, thickening of the lamella epithelium and fusion of the secondary lamellae in the histology of the gills and these are the resultant effects of respiratory stress. Also in *H. bidorsalis* juveniles exposed to varying degree of 2-[(Phosphonomethyl) amino] acetic acid toxicity, the liver showed Slight degeneration and vacuolation

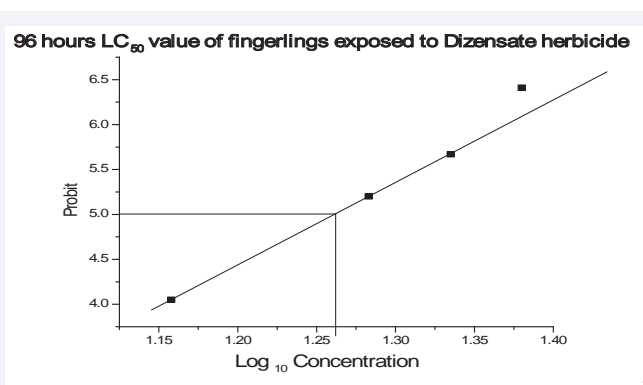


Figure 1 Effect of 2-[(Phosphonomethyl) amino] acetic acid (glyphosate herbicide) on *H. bidorsalis* juveniles.

Table 3: Histological alterations in organs of *Heterobranchus bidorsalis* juveniles subjected to 2-[(Phosphonomethyl) amino] acetic acid (glyphosate herbicide).

Concentration g/L	Gills	Liver
0	Normal gill architecture. Central cartilage and the afferent filament artery are evident	Normal hepatocellular architecture.
9.6	Mild degeneration in the gill architecture.	No noticeable lesion. Hepatocyte showing bile duct and hepatic artery.
14.4	Atrophy of the filament artery which transport blood to the lamellae.	Slight degeneration and vacuolation of the hepatocyte.
19.2	Distorted lamellar epithelium	Evidence of inflammation of the liver cells with cellular infiltration of hepatocellular parenchyma.
21.6	Epithelia proliferation/erosion of gill filaments and lamellae.	Evidence of fatty acid degeneration.
24.0	Necrosis of the gill cells	Severe hepatic damage as a result of hepatotoxicity of glyphosate.

of the hepatocyte, evidence of inflammation of the liver cells with cellular infiltration of hepatocellular parenchyma, evidence of fatty acid degeneration and finally severe hepatic damage, this is as a result of hepatotoxicity of glyphosate. In an akin study by Ayoola, on the effects of glyphosate on juvenile African catfish, *Clarias gariepinus*, glycogen vacuolation, fatty infiltration, hemosiderosis and congested central vein at a lower concentrations of 1.9 to 9 mgL⁻¹ while at higher concentration, severe infiltration of leukocytes, pyknotic, hepatic necrosis, severe necrotic haemorrhage and vacuolation

CONCLUSION

The results of this study revealed that 2-[(Phosphonomethyl) amino] acetic acid (glyphosate) herbicide is toxic to fish organs and causes histological alterations in the gills which is an interface for the exchange of gases and also in the liver, which plays a central role in the metabolism of foreign substances and it's the center

of deamination of toxic substances [19]. They both had different levels of debasement after exposure to 2-[(Phosphonomethyl) amino] acetic acid. This further establishes the illfare effects this toxicant has on *Heterobranchus bidorsalis* juveniles. Therefore, thoughtless discharge of 2-[(Phosphonomethyl) amino] acetic acid into water bodies should be frowned at.

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